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<b>(21) International Application Number:</b> PCT/IL96/00118 <b>(22) International Filing Date:</b> 26 September 1996 (26.09.96)  <b>(30) Priority Data:</b> 115465 29 September 1995 (29.09.95) IL  <b>(71) Applicant (for all designated States except US):</b> YEDA RESEARCH AND DEVELOPMENT CO., LTD. [IL/IL]; Weizman Institute of Science, P.O. Box 95, F-76100 Rehovot (IL).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SHINITZKY, Meir [IL/IL]; 20 Derech Haganim, 46544 Kfar-Shmaryahu (IL). DECKMANN, Michael [DE/FR]; 24, rue de Kreutzberger, F-68500 Guebwiller (FR).  <b>(74) Agent:</b> GONEN, Bossmat; Reinhold Cohn and Partners, P.O. Box 4060, 61040 Tel-Aviv (IL).		<b>(81) Designated States:</b> AU, BR, CA, IL, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ASSAY FOR THE DIAGNOSIS OF DEMENTIA  <b>(57) Abstract</b> <p>An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type in an individual is provided. It comprises the steps of obtaining a sample from said individual being a blood sample, a platelet-containing fragment thereof, or a fragment containing platelet associated antibodies (PAA) detached from the platelets, and then determining the level of a platelet associated antibody against a 75 kD platelet-protein in said sample. A level higher than that of a control sample indicates that said individual has a high likelihood of having multi-infarct dementia or dementia of the Alzheimer type.</p> <div data-bbox="600 1155 1380 1680"></div>		

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## ASSAY FOR THE DIAGNOSIS OF DEMENTIA

### FIELD OF THE INVENTION

The present invention relates to an assay for the diagnosis of multi-infarct dementia (MID) and dementia of the Alzheimer type (DAT).

### 5 BACKGROUND OF THE INVENTION

Dementia of the Alzheimer type (DAT) is the most common cause of dementia in the elderly (Evans, D.A., *et al.*, *J. Am. Med. Assoc.*, 262:2551 (1989)).

10 The pathogenesis of DAT involves accumulation of amyloid and paired helical filaments (PHF) in cerebral tissues as well as synaptic loss, both leading to neuronal death which eventually results in mental deterioration (Massimo Tabaton, M., *Alzheimer's Disease Update*, 10:249 (1994)).

15 The clinical diagnosis of DAT is confounded by other dementing diseases, particularly those common in the elderly, having similar clinopathological features. One such dementia which cannot be effectively separated by diagnosis from DAT is multi-infarct dementia (MID) in which cerebral tissue damage seems to be caused mainly by breakage of small cerebral vessels leading to clinopathologic results which are similar to those detected in DAT.

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At least some forms of DAT have a genetic etiology and, recently, three different genes have been reported in which mutations related to this disease were identified (Levy-Lahad, E., *Science*, 269:973 (1995)). Identification of such genes will no doubt contribute to the understanding of the pathogenesis of the disease, but as of today, these findings are not yet utilized for diagnosis of DAT.

The existence of elevated levels of auto antibodies on blood platelets of various demented patients has been previously reported (Shinitzky, M. *et al.*, *Am. N. Y. Acad. Sci.*, 621:205-217 (1991)). However, these findings, as well, could not be used for diagnosis of MID or DAT.

The diagnosis of DAT today is carried out by an exclusion process, i.e. by excluding possible other dementias and remaining with a high probability that the dementia in question is indeed DAT. Confirmation of such diagnosis is possible only by performing postmortem histopathological examination of brain tissues. Therefore, in actuality, no diagnostic assay for diagnosing MID and DAT is yet available.

Neuronal cell death, which occurs in MID and DAT patients, is a non reversible phenomena. Therefore, it is clearly highly desirable to provide an assay for early diagnosis of MID and DAT on the basis of which it would become possible to detect individuals having a high risk of developing these diseases. Such individuals could then be treated with various medications intended to delay or, if possible, to prevent, the clinical processes causing the onset of these diseases.

## **SUMMARY OF THE INVENTION**

The present invention is based on the surprising finding that patients suffering from multi-infarct dementia (MID) and dementia of the Alzheimer type (DAT), display a high level of a 75 kD platelet protein and/or a high level of platelet-associated antibodies against said protein as compared to normal subjects.

This surprising finding paved the way to the development of an assay for the detection of MID and DAT by detecting the levels of the 75

kD platelet protein, the level of platelet-associated antibodies against this protein or the levels of both, and comparing said level or levels to those of a normal control, a higher level indicating a likelihood that the tested individual has MID and/or DAT.

5           According to one aspect of the invention termed "*the antibody aspect*" the present invention provides an assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type in an individual comprising the steps of:

- 10           i. obtaining a sample from said individual being a blood sample, a platelet-containing fragment thereof, or a fragment containing platelet associated antibodies (PAA) detached from the platelets;
- ii. determining the level of a platelet associated antibody against a 75 kD platelet protein in said sample, a level higher than that of a control sample indicating that said individual has a high  
15           likelihood of having multi-infarct dementia and dementia of the Alzheimer type.

          According to another aspect of the present invention termed "*the protein aspect*" the present invention provides an assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type an individual  
20           comprising the steps of:

- i. obtaining a sample from said individual being a blood sample or a platelet-containing fragment thereof;
- ii. determining the level of a 75 kD platelet protein in said sample, a level higher than that of a control sample indicating that said  
25           individual has a high likelihood of having multi-infarct dementia and dementia of the Alzheimer type.

          According to yet another aspect of the invention termed "*the antibody and protein aspect*" the invention provides an assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type in  
30           an individual comprising the steps of:

- i. obtaining a sample from said individual being a blood sample or a platelet-containing fragment thereof;

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- ii. determining the level of a platelet-associated antibody against a 75 kD protein and the level of a 75 kD platelet protein in said sample, levels being higher than the corresponding two levels of a control sample indicating that said individual has a high likelihood of having multi-infarct dementia and dementia of the Alzheimer type.

The assay of the invention is suitable for detecting both multi-infarct dementia, and dementia of the Alzheimer type since, as explained above, the clinical symptoms of the two types of dementia are usually indistinguishable. As acceptable today, all elderly patients suffering from DAT are believed to be also effected by MID.

The sample of the tested individual may be a blood sample such as serum; a platelet-containing fraction thereof such as platelet rich plasma (PRP) obtained by treatment of the blood with an anti-coagulant e.g. heparin or sodium citrate and centrifugation after which platelets may be obtained from the pelleted fraction; or, in the antibody aspect of the invention, a blood sample fraction containing PAA detached from the obtained platelets.

According to the antibody aspect of the invention where the level of the PAA against the 75 kD platelet protein is to be determined, the antibodies are obtained by centrifugation of PRP, incubation of the pelleted platelets with glycine HCl buffer pH 2.5-3.0 at room temperature and centrifugation, thus obtaining the PAA-containing supernatant. The level of the PAA against the specific 75 kD platelet protein level is then determined as will be explained hereinbelow.

According to the protein aspect of the invention, the platelet proteins are obtained by separation of the platelets from the sample, for example, by obtaining PRP as explained above, and then solubilizing the platelets and separating their proteins by methods well known in the art such as, for example, by polyacrilamide gel electrophoresis (PAGE) by affinity chromatography, high-performance liquid chromatography (HPLC) etc.

The level of the 75 kD protein obtained is determined as will be explained hereinbelow.

According to the antibody and protein aspect of the invention, both the PAA and platelet proteins are separately obtained from the sample and the level of the PAA against the 75 kD platelet-protein as well as the level of the protein itself are separately determined.

An example of a method for determination of the level of the PAA against the 75 kD platelet-protein is by immobilization of the 75 kD protein, or an antibody binding fragment of said protein, by their binding to a solid support such as nitrocellulose, or various kinds of inert beads and then contacting the immobilized proteins with the PAA containing sample under conditions allowing antibody-antigen binding (Shinitzki *et al.*, *ibid.*). Unbound antibodies are removed and the level of bound antibodies is detected, for example, by the use of anti-human immunoglobulin antibodies or an antigen binding fragment thereof linked to a marker. Such a marker may be for example, a radioactive group; a fluorescent group; an enzyme that can catalyze a reaction yielding a detectable product such as, for example, horseradish peroxidase (HRP) and alkaline phosphatase; a biotin group that can be detected by streptavidin, etc.

Another example of a method for determining the level of PAA against the 75 kD platelet-protein is by use of anti-idiotypic antibodies raised by immunizing an animal with a purified preparation of PAA. According to this method, the PAA antibodies of the sample are immobilized onto a solid support, for example, by their binding to anti-human immunoglobulin antibodies. The obtained anti-idiotypic antibodies are then contacted with the immobilized PAA under conditions allowing antibody-antibody binding and unbound antibodies are removed. The anti-idiotypic antibodies may be directly labelled with detectable markers such as radioactive, fluorescent or enzymatic groups. Alternatively, the level of the bound anti-idiotypic antibodies may be determined by contacting the first unlabelled bound anti-idiotypic antibodies with a secondary labelled species

specific anti-immunoglobulin antibody (directed against the species of the anti-idiotypic producing animal).

5 The separation and immobilization of the platelet-proteins can be carried out by electrophoretic separation of the proteins on a polyacrylamid gel, for example, a 7.5-15% acrylamid gradient gel, and then the transfer of the separated proteins onto a nitrocellulose sheet. The detection of the 75 kD platelet-protein can then be achieved by contacting the protein containing sheet with anti-75kD platelet-protein PAA as described above, (i.e. by the technique known as "western blotting").

10 The 75 kD platelet protein may also be detected, for example, by use of affinity binding assays wherein the binding molecules used are various molecules such as receptors, enzymes, etc., capable of specifically binding and recognizing the 75 kD platelet protein.

15 Once the level of the antibody, the protein or both the antibody and the protein have been determined, their level is compared to the corresponding level or levels of the antibody and/or protein in a control sample.

A control sample may be blood samples of the type defined above pooled from several individuals which do not feature symptoms of dementia. It is essential not to use as a control a sample from a single person even if he is considered healthy, since about 25% of the population will eventually suffer from dementia and it is believed that the onset of the disease occurs decades before the clinical symptoms of dementia appear. It may well be, therefore, that during this symptom free period the level of the 75 kD platelet-protein or the level of PAA against it already begin to change

25 Alternatively, it is possible to use as a control sample a pool of samples from individuals in which no anti 75 kD platelet-protein PAA were detected.

A high determined level of the anti 75 kD platelet-protein PAA or 75 kD platelet-protein itself, or both, in a tested individual as compared to the corresponding level of the antibody and/or protein in the control sample, indicates a likelihood of the individual having MID or DAT.

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Experimental results obtained in accordance with the present invention showed that, while essentially all dementia patients show raised level of the anti 75 kD platelet-protein antibody, the protein itself or both, so did a small percentage of healthy individuals. While this may be a false positive result, it may also be explained by the fact that the level of the 75 kD platelet-protein or the PAA antibody against it is indicative of future development of dementia in an individual. Thus, a positive result indicates a high likelihood of having MID or DAT, either given the person is currently suffering from these types of dementia or given he will be effected by these types of dementia later in life.

The present invention further comprises a kit for use in the assays of the invention comprising substances and reagents required for determining the level of the 75 kD platelet-protein, the PAA against the 75 kD platelet-protein or both, as the case may be.

The invention will now be illustrated with reference to some non-limiting examples.

## BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows a photograph of a Western blot in which PAA from demented patients (Fig. 1A) from elderly normal subjects (Fig. 1B) and from normal young to middle aged subjects (Fig. 1C) were incubated with normal human platelet-proteins.

Fig. 2A shows a photograph of a Western blot prepared from a pool of platelets derived from healthy subjects probed with PAA from demented patients.

Fig. 2B shows a photograph of a Western blot of a pool of platelets derived from demented patients probed with PAA from demented patients.

Fig. 3A shows a photograph of a Western blot of a pool of platelets derived from normal subjects probed with PAA from demented patients.

Fig. 3B shows a photograph of a Western blot prepared from a pool of platelets derived from demented patients and probed with PAA from normal subjects.

Fig. 4 shows a photograph of a blot showing platelet-proteins of a pool of platelets derived from 60 blood donors after preparative isoelectric focusing of the proteins (see above) and incubation with anti-75 kD protein PAA.

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## DETAILED DESCRIPTION OF THE INVENTION

### General Procedures

#### i. Patients and control subjects

Young (< 65 years) and healthy volunteers, old (> 65)) non-demented volunteers and old (> 65 years) demented patients participated in this study. The patients and the old non-demented volunteers were members of the Geriatric Hospital, Pardes Hanna, Israel, whereas the young volunteers were staff members of the Geriatric Hospital, Pardes Hanna, Israel and The Weizmann Institute of Science, Rehovot, Israel. For the demented patients participating in this study, all other organic reactions except for multi-infarct dementia (MID) and dementia of the Alzheimer type (DAT) were excluded.

#### ii. Preparation of Platelet Rich Plasma (PRP)

Venous blood was drawn in the morning using heparin as anticoagulant. PRP was obtained from full blood by centrifugation (100 x g. for 15 mins.) at room temperature (20-24°C). The number of platelets in the PRP were scored microscopically.

#### iii. Isolation of Platelet Associated Antibodies (PAA) from platelets

4 to 15 ml of PRP were centrifuged (2000 x g for 20 mins) at room temperature. The supernatant was discarded and the pellet was resuspended in 5 ml PBS/10 mM EDTA and centrifuged again. This step was repeated three times. For the removal of PAA from the pelleted platelets, the platelets were resuspended in 1 ml 150 mM glycine/HCl buffer, pH 2.7, for 10 mins. at room temperature. The platelets were then pelleted by centrifugation (2000 x g for 15 mins.) and the PAA containing superna-

tant was collected and immediately neutralized to pH 7 with the appropriate amount of a saturated solution of  $\text{Na}_2\text{PHO}_4$ .

**iv. Polyacrylamide gel electrophoresis (PAGE)**

5           Platelets were derived from PRP by three washings with PBS/10 mM EDTA and separated by 10% PAGE according to their molecular weights as described in Laemmli, U.K., *Nature.*, 227, 680 (1970) and in Shinitzky *et al.*, *ibid.*

10   **v. Western blot**

          For the electrophoretic transfer of proteins, from the gel (see (iv) above) onto a nitrocellulose sheet, the 10% PAGE was placed between sheets of nitrocellulose or PVDF membranes, Whatmann filter paper No. 4 and pads as holders. The transfer was performed in a Western blot cell  
15 (BioRad), filled with transfer buffer. Two transfer buffers were used: for transfer in two hours by 750 mA at 10°C, the buffer consisted of 0.025M Tris, 0.192M glycine, 0.1% SDS and 20% methanol. For transfer overnight (about 12 hours) by 100 mA at 10°C, the buffer consisted of 10 mM Caps, pH 11 and 10% methanol. The efficiency of the protein transfer was  
20 monitored by a nitrocellulose sheet stained with the reversibly-binding dye Poinceau S. After removal of the Poinceau S stain with double distilled water, the nitrocellulose sheet was air-dried, cut into 0.3 cm wide strips and stored dry at room temperature until use.

25   **vi. Western blot analysis**

          The proteins which were transferred into the nitrocellulase sheet ((v) above) were probed as follows: All incubation steps were carried out at room temperature and under constant shaking (Belco Rocking Table, speed setting 4) in BioRad Incubation Trays. The strips were first incubated  
30 for 5 mins. in 1 ml incubation buffer (60 mM citric acid, 90 mM  $\text{Na}_2\text{PHO}_4$ , 0.3% Tween 20, 200 mM NaCl, pH 7.7) in order to wet and block the strips. Then, the sample (e.g. 0.2 ml PAA, 0.1 ml plasma) was added and

- 10 -

incubated for 14 hours. After the incubation, the strips were washed 3 times with 1 ml incubation buffer and one time with 1 ml peroxidase buffer (200 mM Tris, 0.3% Triton X-100, 10 mM phenol, 2 mM  $\text{CaCl}_2$ , pH 8.0 for 15 mins. The strips were then incubated with goat anti-human  $\text{F}_c$ -IgG covalently linked to horse radish peroxidase (Bio Makor, Israel) in a dilution of 1:250 in 1 ml per strip for 2 hours. The strips were then washed 3 times with 1 ml peroxidase buffer and one time with 1 ml double distilled water for 10 mins. Bound peroxidase was visualized by incubating the strips with 1 ml color reagent (12 mg 4-chloronaphthol, 4 ml methanol, 20 ml PBS, 0.005 ml 30%  $\text{H}_2\text{O}_2$ ) for 30 to 60 mins. The color reaction was stopped by washing the strips with double distilled water.

#### vii. Preparative isoelectric focusing

Platelet Rich Plasma from 60 blood bank donors (blood group O) were pooled and extensively washed with PBS/10 mM EDTA until the supernatant was free of plasma proteins. The platelets were then solubilized with 1% NP40 in DDW. The unsolubilized material was pelleted by centrifugation at 10,000 g for 15 mins. at 4°C. The unsolubilized was then repeatedly treated with 1% Triton X-100 in DWW until the supernatant became poor in protein. All supernatants were pooled and isoelectric focused in a ROTOFOR apparatus (BioRad) according to the instructions of the manufacturer. A pH gradient of 1.5 to 12.5 was generated using ampholyte 3/10 from BioRad. After focusing, the fractions were analyzed for pH, protein pattern and relevant proteins.

#### Example 1

PAA from 9 demented patients, 10 old but normal subjects and 9 young to middle aged normal subjects were incubated with normal human platelet proteins, separated by gel electrophoresis and blotted onto nitrocellulose as described above. Visual detection of bound PAA was carried out as described above. The results are shown in Fig. 1, wherein molecular

weight markers from BioRad were used and the arrows indicate the positions of the 66 kD, 97 kD and the 200 kD markers.

With PAA from the demented patients, a protein with an apparent molecular weight of about 75 kD was detected in all cases (Fig. 1A). An additional protein with an apparent molecular weight of about 110 kD was detected in three cases (Fig. 1A). The 75 kD band could only faintly be detected in 3 out of 10 old non demented subjects (Fig. 1B) and 3 out of 9 young middle aged normal subjects (Fig. 1C). As explained above, this indication may not merely present a false positive but may indicate a tendency of acquiring MID or DAT later in life.

## Example 2

Platelets from healthy and normal subjects whose PRP did not contain detectable amounts of PAA against the 75 kD protein, determined as described in Example 1, were pooled. The platelets from demented patients were also separately pooled. The proteins of each of these two pools were separated by gel electrophoresis and blotted onto nitrocellulose as described above. The blot prepared from platelets of healthy subjects will be referred to hereinafter as the "*normal*" blot and the blot prepared from platelets of the demented patients will be referred to as the "*dementia*" blot. The "*dementia*" Western blot was then probed with PAA from demented patients, whereas the "*normal*" Western blot was probed with PAA from non demented subjects as described above. Under these conditions, the 75 kD band was not detected on the "*normal*" Western blot (Fig. 2A). However, this band became strongly visible, when PAA from demented patients was used to probe the "*dementia*" Western blot (Fig. 2B).

In order to determine whether the 75 kD protein is dementia specific, a cross-over experiment was conducted. The "*dementia*" Western blot was probed with PAA from normal subjects and the "*normal*" Western blot was probed with PAA from demented patients as described above. Surprisingly, the PAA from demented patients could not detect the 75 kD

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protein in Western blot of normal subjects (Fig. 3A)., the PAA from normal subjects did not at all or only faintly detect this protein in the Western blot of demented patients (Fig. 3B).

5        These results indicate that an autoantibody against the 75 kD protein exists in demented patients and that the 75 kD protein itself seems newly generated in demented patients and is absent in platelets from normal subjects.

### Example 3

10        Platelets from 60 blood donors (blood group 0) were isolated from platelet rich plasma as described above. The pooled platelets were solubilized and separated by isoelectric focusing as described above. The proteins of the different fractions ranging from pH 1.5 to 12.5, were separated by gel electrophoresis, blotted and screened for the 75 kD protein  
15 as described above.

      The result of this experiment are shown in Fig. 4. As can be seen, the 75 kD protein could be found in the pool from 60 blood donors as expected from a statistical point of view, since 25% of the elder people will suffer from dementia.

20        Fig. 4 also shows that the 75 kD protein has a pI between pH 7 to pH 9, which indicates that this protein exists in different isoforms and/or that there is a family of several proteins with the same molecular weight but different pI involved.

**CLAIMS:**

1. An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type in an individual comprising the steps of:
  - 5 i. obtaining a sample from said individual being a blood sample, a platelet-containing fragment thereof, or a fragment containing platelet associated antibodies (PAA) detached from the platelets;
  - ii. determining the level of a platelet associated antibody against a 75 kD platelet-protein in said sample, a level higher than that of  
10 a control sample indicating that said individual has a high likelihood of having multi-infarct dementia or dementia of the Alzheimer type.
2. An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type an individual comprising the steps of:
  - 15 i. obtaining a sample from said individual being a blood sample or a platelet-containing fragment thereof;
  - ii. determining the level of a 75 kD platelet-protein in said sample, a level higher than that of a control sample indicating that said individual has a high likelihood of having multi-infarct dementia  
20 and dementia of the Alzheimer type.
3. An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type in an individual comprising the steps of:
  - i. obtaining a sample from said individual being a blood sample or a platelet-containing fragment thereof;
  - 25 ii. determining the level of a platelet-associated antibody against a 75 kD protein and the level of a 75 kD platelet-protein in said sample, levels being higher than the corresponding two levels of a control sample indicating that said individual has a high likelihood of having multi-infarct dementia and dementia of the  
30 Alzheimer type.
4. An assay according to Claims 1 or 3, wherein the determination of the level of the antibody comprises:

- (a) immobilizing the 75 kD platelet-protein or an antibody-binding fragment thereof, onto a solid support;
  - (b) contacting the sample with the immobilized protein under conditions allowing antibody-antigen binding;
  - 5 (c) removing unbound antibodies from the solid support; and
  - (d) determining the level of antibody bound onto the solid support.
5. An assay according to Claim 4, wherein the determination of step (d) comprises adding to the bound antibodies anti-human immunoglobulin antibodies conjugated to a detectable marker.
- 10 6. An assay according to Claims 2 or 3, wherein determination of the 75 kD platelet protein level comprises:
- (a) immobilizing the sample's proteins onto a solid support;
  - (b) contacting the immobilized proteins with platelet-associated antibodies capable of specifically binding to the 75 kD protein or with
  - 15 antigen-binding fragments thereof of said antibodies;
  - (c) removing unbound antibodies from the solid support;
  - (d) determining the level of bound antibodies.
7. An assay according to Claim 6, wherein the determination of step (d) comprises adding to the bound antibodies anti-human immunoglobulin antibodies conjugated to a detectable marker.
- 20 8. An assay of Claim 6, wherein the immobilization of step (a) comprises electrophoretically separating the sample's proteins on a polyacrylamide gel and then immobilizing said separated protein on to a nitrocellulose support.
- 25 9. A method according to Claim 8, wherein the gel is a 7.5-15% acrylamide gradient gel.
10. A kit for use in the assay of any one of the preceding claims.



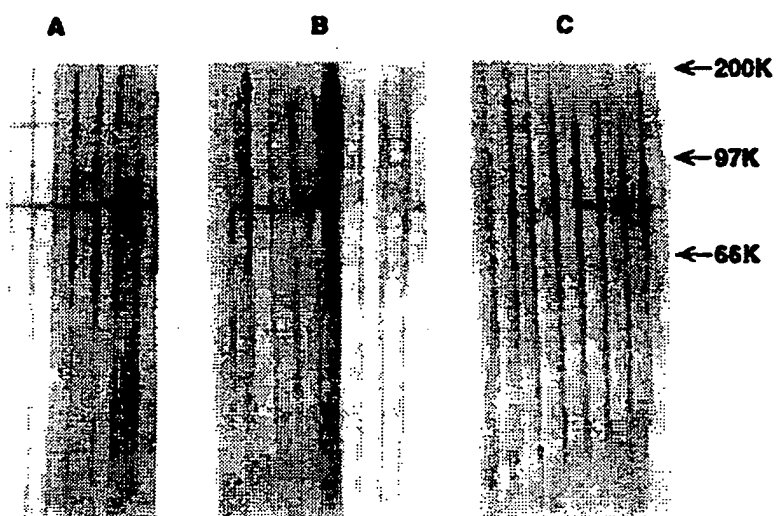
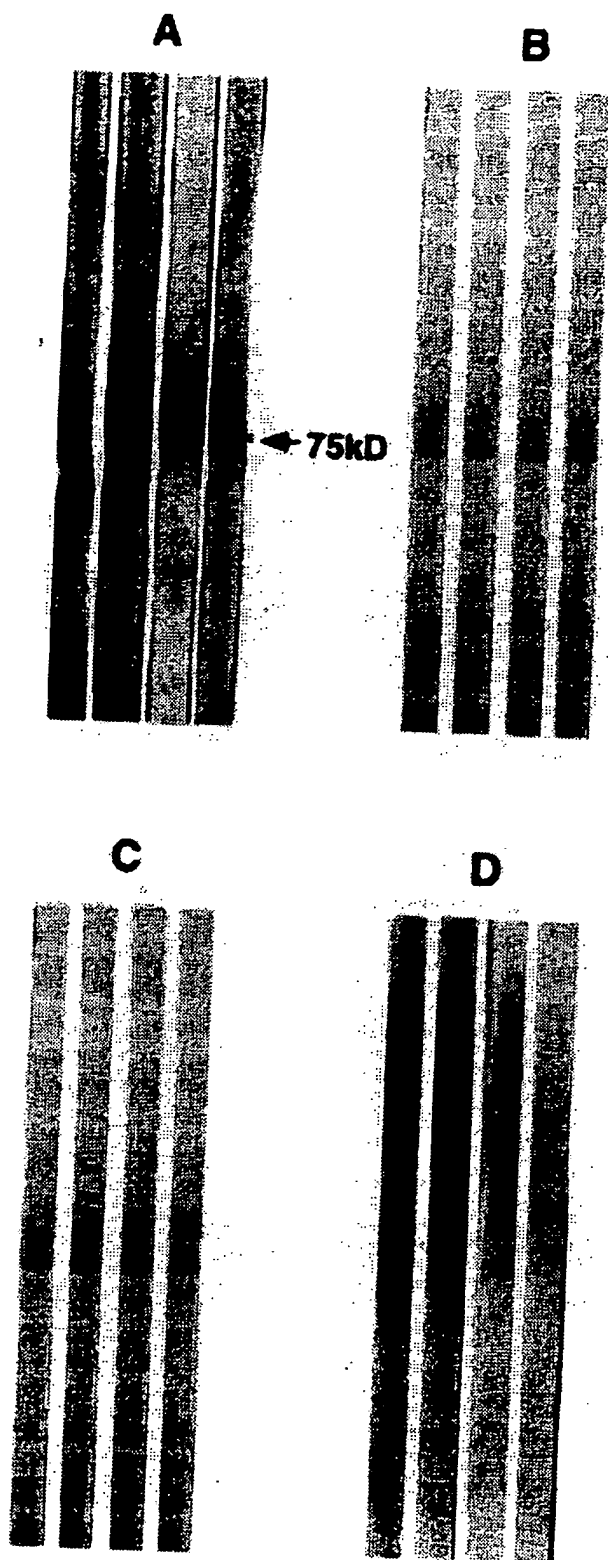


FIG.1

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**FIG.2**  
**SUBSTITUTE SHEET (RULE 26)**

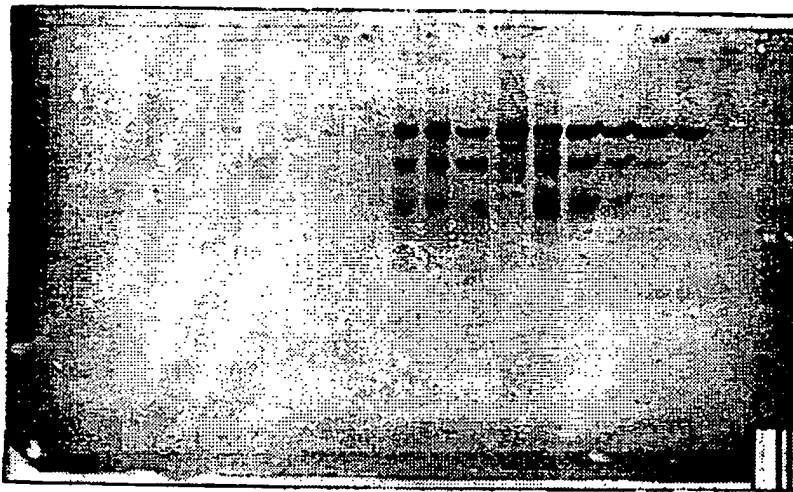


FIG.3

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/IL 96/00118

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 G01N33/68 G01N33/564 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 G01N

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 15773 A (OXFORD VIROLOGY PLC) 17 October 1991 see claims; figure 1	1-10
Y	AN. N.Y. ACAD. SCI., vol. 621, 1991, pages 205-217, XP002024437 SHINITZKY, M. ET AL.: "Platelet autoantibodies in dementia and schizophrenia - possible implication for mental disorders" cited in the application see the whole document	1-10
Y	WO 95 12685 A (YEDA RES & DEV ;RYCUS AVIGAIL (IL); SHINITSKY MEIR (IL)) 11 May 1995 see the whole document	1-10

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 23970 A (YEDA RES & DEV ;RYCUS AVIGAIL (IL); SHINITZKY MEIR (IL); DECKMANN) 8 September 1995 see the whole document ---	1-10
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